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TITLE: EGFR Pathway Modulation in Ductal Carcinoma *in Situ* of the Breast

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13. ABSTRACT (Maximum 200 Words)

We proposed a clinical trial to study the modulation of the EGFR pathway in DCIS. Eligible patients have either a mammogram highly suspicious for DCIS or a recent diagnosis of DCIS through a core biopsy. Subsequently, patients whose DCIS expresses EGFR are randomized to receive an EGFR inhibitor (Iressa) or placebo for 3 weeks prior to surgery. The protocol was generated, approved by the IRB and an efficient system for specimen collection was implemented. Since EGFR expression was a crucial requirement to enter the study we developed the immunohistochemical assay for the detection of the EGFR and P-EGFR proteins. We conducted an IRB-approved, retrospective study of DCIS cases diagnosed by **core biopsy** at our institution. A total of 42 cases were studied for EGFR and P-EGFR. We showed that EGFR expression is present in 18.6% of DCIS and correlates with high grade. P-EGFR expression was low at 7%. We also conducted a retrospective study using **paraffin embedded tissues.** 50 cases of DCIS were obtained from the Cooperative Breast Cancer Tissue Resource (CBCTR). IHC studies were performed for EGFR, P-EGFR, Ki67, p27, P-ERK, P-AKT. We confirmed that the rate of EGFR positivity in DCIS is 20%. P-EGFR expression was 5%.

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Table of Contents

Annual Summary

Introduction

We proposed a clinical trial to study the modulation of the Epidermal Growth Factor Pathway (EGFR) in Ductal Carcinoma in Situ (DCIS). Eligible patients have either a mammogram highly suspicious for DCIS or a recent diagnosis of DCIS through a core biopsy. If the diagnosis of DCIS is confirmed upon pathologic review, the tissue is subsequently analyzed for the presence of the EGFR receptor by immunohistochemistry. Subsequently, patients whose DCIS expresses EGFR are randomized to receive an EGFR inhibitor (Iressa) or placebo for 3 weeks prior to surgery.

Body

Task 1. Develop protocol for patient enrollment/specimen collection. Formal courses. Months 1-3

I generated the clinical protocol for enrollment of patients with DCIS, including eligibility criteria, exclusion criteria, treatment plan, statistical endpoints, adverse events expected, consent form. The protocol was submitted to the Internal Review Board (IRB), IRB approval was obtained and the protocol was open for enrollment. I worked with our pathology department, specifically Dr Simpson and Dr Sanders to develop an efficient system for specimen collection with special attention to protecting patient confidentiality. Once the tissue is collected from patient it is delinked from identifiers and re-labeled in numerical order. Only the PI has the key to patient identification.

Task 2. Patient enrollment, specimen collection and laboratory analysis. Months 3-18

Since presence of EGFR expression was a crucial requirement to enter the study we proceeded to develop the immunohistochemical assay for the detection of the EGFR protein and phospho-EGFR (P-EGFR protein). Several primary antibodies, concentrations and methods for antigen retrieval were tested. Placenta was used to optimize the staining for EGFR since placenta is known to express high levels of EGFR. For P-EGFR we obtained a sample of head and neck squamous carcinoma from Dr Frederico Rojo (Spain) that was known to express P-EGFR. We also tested several methods for EGFR detection, using detection kits such the Labeled Streptavidin Biotin LSAB plus kit, the Envision plus kit and the Catalyzed Signal Amplification (CSA) kit (all from Dako, Carpinteria, CA). The LSAB+ detection system (using labeled streptavidin biotin) had accurate sensitivity and specificity for EGFR while the CSA assay (using tyramide) was used for P-EGFR detection.

Subsequently we conducted a retrospective survey of DCIS tissues to evaluate the staining on archival paraffin embedded tissue. After IRB approval we obtained 42 cases of DCIS diagnosed at Vanderbilt by core biopsy between January 2000 to present. All cases were stained for EGFR and P-EGFR and nine of these cases were also evaluated for HER2-neu, Ki67, and p27 expression.

Scoring:

EGFR and P-EGFR: Tumor was considered positive for either marker when unequivocal membrane immunoreactivity in minimal of 10% of DCIS cells was identified. Staining was scored as 0, 1, 2 or 3+ using criteria by Goldstein et al (Cancer, 2001). The presence and localization of cytoplasmic staining for P-EGFR was also recorded.

HER2/neu: HER-2 status was assessed using the FDA approved HercepTest and grading system as follows: negative, 0 or 1+, weakly positive 2+, strongly positive 3+ strictly following the scoring system recommended by the manufacturer.

Ki 67, p27: These assays were considered positive when nuclear immunoreactivity was identified in >5% of DCIS. The presence and localization of cytoplasmic staining for p27 was also recorded.

Table 1.

DCIS	High Grade	Low and Intermediate	Total
EGFR +	8	0	8
EGFR -	19	15	34
Total	27	15	42

EGFR expression was detected in 8/42 cases (19%) (Table 1). Staining intensity was 1+ in three cases and 2+ in 5 cases. The nuclear grade was high in 27 cases, intermediate in 10, and low in 5. All cases showing EGFR positivity were high grade DCIS. Four of the eight DCIS positive cases that were positive for EGFR were associated with invasive mammary carcinoma that was also EGFR positive. Of the 42 cases studied only 3 DCIS cases were P-EGFR positive. A Pearson's chi square test suggested an association between high-grade DCIS and EGFR positivity p=0.053.

To date, 9 of these cases were also analyzed for Her2 neu ,Ki67, and p27,as shown below in Table 2.

Table 2.

DCIS cases n=9	Low Grade	Intermed Grade	High Grade
	2	5	2
Her2 positivity (nr cases, intensity)	1(1+)	3 (1+ and 2+)	2(3+)
EGFR	0	1 (3+)	2(3+)
P-EGFR	0	1 (1+)	1(3+)
Ki67	<5%	5-20%	>20%
P27 staining percent and localization N=nuclear C=cytoplasmic	60-80% (2) N, C	60-80% (3)	15-20% N, C (1)

We presented this data at the American Society of Clinical Oncology Meeting 2003, New Orleans, LA, in a poster session. This retrospective study suggested that the EGFR expression in DCIS is much lower than reported in the literature. Suo, Ottestad and Nesland published the only paper evaluating the expression of EGFR family members in DCIS in 2001. They studied paraffin-embedded sections from 40 cases of pure DCIS of the breast. Using IHC they found that 48% of cases were reactive for EGFR, 63% for ERBB2 and 78% for erbB3, 95% for erbB4. One reason why their results may be different from ours could be the fact that they used a polyclonal antibody from Oncogene at 1:50 dilution, which is different from the antibody we used in our study.

To determine weather the low frequency of EGFR expression was due to the fact that we used core biopsies rather than full paraffin embedded tissue for surgery, we decided to study tissue obtained at surgery. Since at our Institution the DCIS tissues were already committed to another study we submitted an application for breast cancer tissue from the Cooperative Breast Cancer Tissue Resource (CBCTR) and obtained 50 cases of pure DCIS with ten 4-micron sections plus an H&E stained section per case. These cases are also evaluated by IHC for EGFR, Ki67, HER2 and p27. We confirmed our initial findings and again found a 20% rate of EGFR positivity suggesting that the frequency of EGFR expression is indeed lower than previously reported.

Given the low frequency of EGFR positivity in our assay, it was clear to us that a very high number of DCIS patients would have needed to be screened in order to find those that are EFGR positive and therefore eligible to enroll on the study. At the same time data from clinical trials using EGFR inhibitors recently showed that the clinical response to EGFR inhibitors did not correlate with the expression of EGFR receptors in the tumors in that some tumors that lacked EGFR expression responded to treatment and while some tumors that did express EGFR receptors did not respond. After opening the study for accrual we identified a number of items that hindered enrollment.

1. Patients did not want o be randomization to a placebo arm
2. Patients were concerned that the duration of treatment (3 weeks) was too long
3. Financial concerns regarding potential out-of pocket costs if any hospitalizations were needed because of toxicity from the investigational drug.
4. Screened patients had no residual calcifications left after the initial biopsy
5. One patient had a connective tissue disease with dry eyes and dry skin, which could have been exacerbated by Iressa.
6. An overall decrease in the number of DCIS cases was seen compared to previous years For all these reasons, we decided to amend the protocol and relax the eligibility criteria to allow more patients to be enrolled on protocol. The following changes were made:

1. We eliminated the placebo arm of the study so that there will be no randomization to an inactive drug and all patients receive investigational drug. The statistical analysis was reevaluated by our statistician Dr Billheimer and it was determined that the EGFR negative patients could serve as the control population substituting for a placebo arm.
2. Since patients were concerned that 21 days of treatment is too long of duration of treatment and also to reduce the risk of potential toxicity, the duration of treatment was shortened to 7-14 days. Data in gastrointestinal studies has shown that 7-14 days of treatment is sufficient to see changes in parameters such as ki67 and MAPK expression
3. We eliminated the requirement for EGFR positivist for enrollment.
4. Expanded the age range to patients older than 18 years of age.
5. Prepared an " advocate newsletter" to disseminate in the breast cancer advocacy groups and boost visibility of the trial and recruitment and a patient " brochure" describing the 5. We listed the trial on the NIH website, with key information and study contact information.

PLANS

We plan to reach out to the patient population eligible for the trial to increase accrual. We have also contacted several institutions for potential partnerships for this study. Among the institutions that have expressed an interest in participating are: H. Lee Moffitt Cancer Center, Tampa, FL (contact Dr Stacy Moulder), Indiana University, Indianapolis, IN (contact Dr Gabriela Matei).

Key Research Accomplishments:

- ASCO Poster Presentation May 2003 New Orleans, LA. **Truica C.I**, Zou Y, Bylow K, Simpson J, Billheimer D, Sanders M. Evaluation of the EGFR pathway in ductal carcinoma in situ (DCIS) of the breast.

We conducted an IRB-approved, retrospective study of DCIS cases diagnosed by **core biopsy** at our institution between January 2000 and December 2001 for which unstained slides were available. A total of 42 cases were studied for EGFR and P-EGFR. Nine of these cases were also evaluated for HER2-neu, Ki67, and p27 expression.

- We showed that EGFR expression is present in 18.6% of DCIS

- Our data shows that EGFR positivity correlates with high grade
- P-EGFR expression was low in our series- 7%

We conducted a retrospective study of EGFR pathway in DCIS using **paraffin embedded tissues.** 50 cases of DCIS with 10 slides per case were obtained from the Cooperative Breast Cancer Tissue Resource (CBCTR). IHC studies were performed for EGFR, P-EGFR, Ki67, P27 P-ERK, P-AKT. We confirmed that the true rate of EGFR positivity in DCIS is 20% . EGFR positivity correlates with high grade. P-EGFR expression was low at 5%.
Manuscript in preparation.

Reportable Outcomes

Research

Truica C.I, Zou Y, Bylow K, Simpson J, Billheimer D, Sanders M. Evaluation of the EGFR pathway in ductal carcinoma in situ (DCIS) of the breast. Poster presentation at the 2003 American Society of Clinical Oncology Meeting, New Orleans, LA.

Publications

Truica C.I, Zou Y, Bylow K, Simpson J, Billheimer D, Sanders M. Evaluation of the EGFR pathway in ductal carcinoma in situ (DCIS) of the breast. Proc Am Soc Clin Oncol 2003, 22: 3488

Arteaga CL, **Truica CI.** Challenges in the development of anti-epidermal growth factor receptor therapies in breast cancer. Semin Oncol. 2004 Feb;31(1 Suppl 3):3-8.

Conclusions

Our results show that in DCIS EGFR expression correlates with grade. Since high grade DCIS is more likely to recur and become invasive. We also found that overall EGFR expression is lower than expected (20%) rather than the published 50%. If we find that EGFR positivist is one of the requirements for patients to respond to EGFR inhibitors, than the number of patients that would benefit from such treatment is lower than previously anticipated.